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(FILE 'HOME' ENTERED AT 18:13:53 ON 05 FEB 2003)

FILE 'MEDLINE, AGRICOLA, CANCERLIT, SCISEARCH, CAPLUS, MEDICONF' ENTERED AT 18:14:02 ON 05 FEB 2003

L1 2908 S I-SCE? OR I-CSM? OR I-PAN? OR I-CEUI OR I-PPO? OR I-CRE? OR I
L2 829 S L1 AND SITE
L3 647 S L2 AND ENDO?
L4 287 S L3 AND INTRON
L5 125 DUP REM L4 (162 DUPLICATES REMOVED)
L6 125 FOCUS L5 1-
L7 29 S L5 AND CHROMOSOME
L8 29 SORT L7 PY
L9 12 S L8 AND (MAMMAL? OR ANIMAL)

=> d an ti so au ab pi l9 1-12

L9 ANSWER 1 OF 12 MEDLINE
AN 97354303 MEDLINE
TI A **site**-specific DNA **endonuclease** specified by one of two ORFs encoded by a group I **intron** in Dictyostelium discoideum mitochondrial DNA.
SO GENE, (1997 May 20) 191 (1) 115-21.
Journal code: 7706761. ISSN: 0378-1119.
AU Ogawa S; Naito K; Angata K; Morio T; Urushihara H; Tanaka Y
AB The second **intron** (DdOX1/2.2) of Dictyostelium discoideum cytochrome oxidase subunit 1/2 fused gene has two free-standing ORF genes (Dd ai2a and Dd ai2b) in a loop, which have similar amino acid sequences and are homologous to aI4 DNA **endonuclease** (**I-SceII**) of Saccharomyces cerevisiae. To elucidate the functions of these ORFs, we cloned the ORFs into an expression vector and introduced the composite vectors into E. coli. The expression of Dd ai2a in E. coli caused growth inhibition and degradation of the E. coli genomic DNA. To determine whether Dd ai2a protein is a homing type DNA **endonuclease**, the ability to cleave the homing **site** of its **intron** in vivo was examined. Dd ai2a cleaved only one strand of intronless DNA sequence at the **site** which coincides with the **I-SceII** cleavage recognition **site**. We suppose that Dd ai2a functions actually as a homing type DNA **endonuclease** in D. discoideum mitochondria by virtue of other factors. To obtain further information about the relationship between the existence of **introns** and the mating system, we carried out in vitro self-splicing assay and polymerase chain reaction analysis using 13 strains of the cellular slime mold.

L9 ANSWER 2 OF 12 MEDLINE
AN 97254482 MEDLINE
TI New ultrarare restriction **site**-carrying transposons for bacterial genomics.
SO GENE, (1997 Mar 18) 187 (2) 273-9.
Journal code: 7706761. ISSN: 0378-1119.
AU Mahillon J; Rode C K; Leonard C; Bloch C A
AB Electrophoretic separation of macrorestriction fragments containing a particular genomic interval has until recently depended on fortuitously placed native rare restriction **sites**. We present new IS10-based transposons carrying the yeast **intron**-encoded **I-SceI** restriction **site** which is absent from most prokaryotic and eukaryotic genomes. Construction of the plasmid vectors containing them is described. Analysis by conventional or Pulsed Field gel electrophoresis of the DNA fragments generated by the **I-SceI** digestion reveals the physical distance between genomic insertions of these transposons: use of the same approach to subdivide the **chromosome** of Escherichia coli K-12 into equivalently sized contiguous/nonoverlapping **I-SceI** fragments is demonstrated. Because coordinates for the loci delimited by their insertions can be readily determined in different isolates by either physical or genetic manipulations, these transposons allow sufficient flexibility for species-wide bacterial genomics.

L9 ANSWER 3 OF 12 MEDLINE
AN 97153471 MEDLINE
TI Group-I **introns** in the cytochrome c oxidase genes of Dictyostelium discoideum: two related ORFs in one loop of a group-I **intron**, a cox1/2 hybrid gene and an unusually large cox3 gene.
SO CURRENT GENETICS, (1997 Jan) 31 (1) 80-8.
Journal code: 8004904. ISSN: 0172-8083.
AU Ogawa S; Matsuo K; Angata K; Yanagisawa K; Tanaka Y
AB The DNA sequences of cytochrome oxidase (subunits 1, 2 and 3) genes of the cellular slime mold Dictyostelium discoideum mitochondria were determined. The genes for subunits 1 and 2 have a single continuous ORF (COX1/2) which contains four group-I **introns**. The insertion **sites** of the two group-I **introns** (DdOX1/2.2 and DdOX1/2.3) coincide with those of fungal and algal group-I **introns**, as well as a liverwort group-I **intron**, in the cytochrome oxidase subunit 1. Interestingly, **intron** DdOX1/2.2 has two free-standing ORFs in a loop (L8) which have similar amino-acid sequences and are homologous to ai4 DNA **endonuclease** (I-**Sce** II) and bi4 RNA maturase found in group-I **introns** of Saccharomyces cerevisiae mitochondrial DNA.. Two group-I **introns** (DdOX1/2.3 and DdOX1/2.4) also have a free-standing ORF in loop 1 and loop 2, respectively. These results show that these group-I **introns** and the intronic ORFs have evolved from the same ancestral origin, but that these ORFs have been propagated independently.

L9 ANSWER 4 OF 12 MEDLINE
AN 95286526 MEDLINE
TI **I-CeuI** reveals conservation of the genome of independent strains of Salmonella typhimurium.
SO JOURNAL OF BACTERIOLOGY, (1995 Jun) 177 (11) 3355-7.
Journal code: 2985120R. ISSN: 0021-9193.
AU Liu S L; Sanderson K E
AB The enzyme **I-CeuI**, encoded by a class I mobile **intron** inserted in the gene for 23S rRNA in Chlamydomonas eugamatos, cleaves a specific 19-bp sequence in this gene. This sequence is present only in the seven genes for rRNA in Salmonella typhimurium and Escherichia coli. Partial digestion with **I-CeuI** of DNA from 17 wild-type strains of S. typhimurium indicates that the **chromosome** of these strains is strongly conserved, for the digestion products closely resemble those of strain LT2. The lengths and order of chromosomal segments are conserved in 15 of the strains; 2 show some rearrangements. XbaI digestion indicated heterogeneity without revealing the genomic structure. Because of conservation of **I-CeuI sites** in genes for rRNA and conservation of the number and locations of these genes, **I-CeuI** provides an excellent tool for the rapid examination of the **chromosomes** of related species of bacteria; differences in the fingerprints indicate the occurrence of chromosomal rearrangements such as insertions or inversions.

L9 ANSWER 5 OF 12 MEDLINE
AN 95198715 MEDLINE
TI Induction of homologous recombination in **mammalian chromosomes** by using the **I-SceI** system of Saccharomyces cerevisiae.
SO MOLECULAR AND CELLULAR BIOLOGY, (1995 Apr) 15 (4) 1968-73.
Journal code: 8109087. ISSN: 0270-7306.
AU Choulika A; Perrin A; Dujon B; Nicolas J F
AB The mitochondrial **intron**-encoded **endonuclease** **I-SceI** of Saccharomyces cerevisiae has an 18-bp recognition sequence and, therefore, has a very low probability of cutting DNA, even within large genomes. We demonstrate that double-strand breaks can be initiated by the **I-SceI endonuclease** at a predetermined location in the mouse genome and that the breaks can be repaired with a donor molecule homologous regions flanking the breaks. This induced homologous recombination is approximately 2 orders of magnitude more frequent than spontaneous homologous recombination and at least 10 times more frequent than random integration near an active promoter. As a consequence of induced homologous recombination, a heterologous novel sequence can be inserted at the **site** of the

break. This recombination can occur at a variety of chromosomal targets in differentiated and multipotential cells. These results demonstrate homologous recombination involving chromosomal DNA by the double-strand break repair mechanism in **mammals** and show the usefulness of very rare cutter **endonucleases**, such as **I-SceI** , for designing genome rearrangements.

L9 ANSWER 6 OF 12 CANCERLIT
AN 96605697 CANCERLIT
TI Repair of DNA double strand breaks in **mammalian** cells by homologous recombination and end-joining mechanisms (Meeting abstract).
SO J Cell Biochem, (1995) Suppl 21A 328.
ISSN: 0730-2312.
AU Jasin M; Rouet P; Smih F
AB To study the repair of DSBs introduced into **mammalian** chromosomal DNA, we have developed expression vectors for rare-cutting, **site**-specific **endonucleases** from S cerevisiae. We used the universal code equivalent of the mitochondrial **intron** -encoded **endonuclease I-Sce** I to build the **mammalian** expression vector, pCMV-**I-Sce** I. The **I-Sce** I sequence was provided by B Dujon, Pasteur Institute. In addition to providing a consensus Kozak sequence for efficient translation, the **I-Sce** I ORF was modified by fusing sequences encoding a nuclear localization signal and a hemagglutinin epitope tag. Our initial assay for in vivo cutting and enhanced recombination measures extrachromosomal recombination, since this form of recombination is very efficient in **mammalian** cells and sensitive to DSBs. The assay utilizes RSVCAT plasmid substrates consisting of overlapping chloramphenicol acetyltransferase (CAT) gene fragments transiently transfected into cells. The RSVCAT plasmids were modified by the insertion of a synthetic **I-Sce** I **site** at the end of the homology repeats and cotransfections were carried out in COS 1 cells. We observed a substantial increase of CAT activity in cotransfections of pCMV-**I-Sce** I with CAT substrates containing the **I-Sce** I **site** but not with plasmids lacking the **site**. Southern analysis verified in vivo cleavage, as well as recombination. Constitutive expression of the **endonuclease** is not toxic to mouse 3T3 cells. These results have been recently published (Rouet P et al, Proc Natl Acad Sci; 91:6064 1994). We have recently observed efficient cutting of introduced chromosomal **I-Sce** I **sites** in 3T3 cells and have found that they are recombinogenic, stimulating gene targeting two to three orders of magnitude. However, they are also repaired efficiently by end-joining mechanisms (Rouet P et al, Mol Cell Biol, in press). Results of these studies were presented. It is expected that expression of rare-cutting **endonucleases** in **mammalian** cells will provide a powerful tool for the analysis of the repair of chromosomal DSBs, as well as for molecular genetic manipulations, such as **chromosome** fragmentation and, potentially, gene-targeting.

L9 ANSWER 7 OF 12 SCISEARCH COPYRIGHT 2003 ISI (R)
AN 97:40515 SCISEARCH
TI Repair of **site**-specific double-strand breaks in a **mammalian chromosome** by homologous and illegitimate recombination
SO MOLECULAR AND CELLULAR BIOLOGY, (JAN 1997) Vol. 17, No. 1, pp. 267-277.
Publisher: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171.
ISSN: 0270-7306.
AU Sargent R G; Brenneman M A; Wilson J H (Reprint)
AB In **mammalian** cells, chromosomal double-strand breaks are efficiently repaired, yet little is known about the relative contributions of homologous recombination and illegitimate recombination in the repair process.;In this study, we used a loss-of-function assay to assess the repair of double-strand breaks by homologous and illegitimate recombination, We have used a hamster cell line engineered by gene targeting to contain a tandem duplication of the native adenine phosphoribosyltransferase (APRT) gene with an **I-SceI** recognition **site** in the otherwise wild-type APRT(+) copy of the gene. **Site**-specific double-strand breaks were induced by

intracellular expression of **I-SceI**, a rare-cutting **endonuclease** from the yeast Saccharomyces cerevisiae. **I-SceI** cleavage stimulated homologous recombination about 100-fold; however, illegitimate recombination was stimulated more than 1,000-fold. These results suggest that illegitimate recombination is an important competing pathway with homologous recombination for chromosomal double-strand break repair in **mammalian** cells.

L9 ANSWER 8 OF 12 SCISEARCH COPYRIGHT 2003 ISI (R)
AN 95:26377 SCISEARCH
TI THE YEAST-**I-SCE**-I MEGANUCLEASE INDUCES **SITE** -DIRECTED CHROMOSOMAL RECOMBINATION IN **MAMMALIAN**-CELLS
SO COMPTES RENDUS DE L ACADEMIE DES SCIENCES SERIE III-SCIENCES DE LA VIE-LIFE SCIENCES, (NOV 1994) Vol. 317, No. 11, pp. 1013-1019. ISSN: 0764-4469.
AU CHOULIKA A (Reprint); PERRIN A; DUJON B; NICOLAS J F
AB Double-strand breaks in genomic DNA stimulate recombination. Until now it was not possible to induce in vivo **site**-directed double-strand breaks in a **mammalian** chromosomal target. In this article we describe the use of **I-Sce** I meganuclease, a very rave cutter yeast **endonuclease**, to induce **site** -directed double-strand breaks mediated recombination. The results demonstrate the potential of the **I-Sce** I system for **chromosome** manipulation in **mammalian** cells.

L9 ANSWER 9 OF 12 CAPLUS COPYRIGHT 2003 ACS
AN 2002:575221 CAPLUS
DN 137:136055
TI Combinatorial expression libraries with individual members of the library containing concatemers of expression cassettes
SO PCT Int. Appl., 124 pp.
CODEN: PIXXD2
IN Goldsmith, Neil; Sorensen, Alexandra M. P. Santana; Nielsen, Soren V. S.; Naesby, Michael
AB Combinatorial gene expression libraries in which individual clones contain large nos. of expression cassettes and methods of constructing such libraries are described. Each member of the library contains a large no. of expression cassettes that are randomly selected from a pool of cassettes during the construction of the library. Individual expression cassettes are flanked by a common pair of restriction **sites** and have the same promoter and terminator for uniform regulation of expression of the cloned inserts. The library of concatemers is created from a library of individual clones. This primary library, typically a cDNA library, has the individual cassette and its flanking restriction **sites** flanked by a second pair of restriction **sites**. The cassettes are released from the library and ligated into concatemers that are then cloned into a vector capable of stabilizing large inserts, esp. artificial **chromosomes**. The variability within the combinatorial library can be increased by using cDNA libraries from multiple sources. Such libraries are useful in discovery of novel or modified metabolic pathways leading to the prodn. of novel compds. for e.g. drug discovery and to the prodn. of known compds. in novel quantities or in novel compartments of the cells. The expression libraries in particular are composed of host cells capable of coordinated and controllable expression of large nos. of heterologous genes in the host cells.

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 2002059296 | A2 | 20020801 | WO 2002-DK55 | 20020125 |

PI
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

L9 ANSWER 10 OF 12 CAPLUS COPYRIGHT 2003 ACS
AN 2002:403935 CAPLUS
DN 136:396983
TI Nucleotide sequence encoding yeast restriction **endonuclease** **I-SceI** and uses in genetic mapping and **site** -directed gene recombination
SO U.S., 84 pp., Cont.-in-part of U.S. 5,792,632.
CODEN: USXXAM
IN Dujon, Bernard; Choulika, Andre; Perrin, Arnaud; Nicolas, Jean-Francois
AB The present invention relates to an isolated yeast DNA encoding the restriction **endonuclease I-SceI**, and use of **I-SceI** for mapping eukaryotic genomes and for in vivo **site** directed genetic recombination. Specifically, the invention relates to a vector comprising a plasmid, bacteriophage, or cosmid vector contg. the DNA sequence of the enzyme **I-SceI**. The invention also relates to E. coli, eukaryotic cells transformed with a vector of the invention, transgenic **animal** with the DNA sequence encoding **I-SceI**. The invention relates to a transgenic organism in which at least one restriction **site** for the enzyme **I-SceI** has been inserted in a **chromosome** of the organism. The invention further relates to methods for gene mapping in yeast **chromosome**, yeast artificial **chromosome**, and cosmids, and **site**-directed insertion of genes.

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| PI US 6395959 | B1 | 20020528 | US 1996-643732 | 19960506 |
| US 5474896 | A | 19951212 | US 1992-971160 | 19921105 |
| US 5792632 | A | 19980811 | US 1994-336241 | 19941107 |

L9 ANSWER 11 OF 12 CAPLUS COPYRIGHT 2003 ACS
AN 2000:553718 CAPLUS
DN 133:160582
TI Gene repair involving homologous recombination induced by in vivo double-stranded cleavage of targeting DNA mediated by chimeric restriction **endonuclease**
SO PCT Int. Appl., 38 pp.
CODEN: PIXXD2
IN Choulika, Andre; Mulligan, Richard C.
AB Methods of modifying, repairing, attenuating and inactivating a gene or other chromosomal DNA in a cell through chimeric restriction **endonuclease** (or meganuclease)-induced homologous recombination are disclosed. 101The method is exemplified by introducing into a cell a vector contg. a targeting DNA homologous to a chromosomal target **sites** and is flanked by specific **sites** for restriction **endonuclease I-SceI** (a Saccharomyces cerevisiae **intron**-encoded rare-cutter **endonuclease** recognizing 18-bp sequence) or meganuclease, and cDNA encoding **I-SceI** or meganuclease. The **I-SceI** **site** is recognized and cleaved in vivo to relase the repair matrix and induce homologous recombination. The method has applications in treating or prophylaxis of a genetic disease in an individual in need.

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| PI WO 2000046386 | A2 | 20000810 | WO 2000-US3014 | 20000203 |
| WO 2000046386 | A3 | 20001214 | | |
| W: AU, CA, JP, US | | | | |
| RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE | | | | |
| EP 1147209 | A2 | 20011024 | EP 2000-908499 | 20000203 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI | | | | |
| JP 2002535995 | T2 | 20021029 | JP 2000-597445 | 20000203 |
| US 2002107214 | A1 | 20020808 | US 2001-917295 | 20010727 |

L9 ANSWER 12 OF 12 CAPLUS COPYRIGHT 2003 ACS
AN 2000:553717 CAPLUS
DN 133:160581
TI Gene repair involving homologous recombination induced by in vivo double-stranded cleavage of targeting DNA mediated by restriction

**endonuclease**

SO PCT Int. Appl., 47 pp.
CODEN: PIXXD2

IN Choulika, Andre; Mulligan, Richard C.

AB Methods of modifying, repairing, attenuating and inactivating a gene or other chromosomal DNA in a cell through restriction **endonuclease** -induced homologous recombination are disclosed. The method is exemplified by introducing into a cell a vector contg. a targeting DNA homologous to a chromosomal target **sites** and is flanked by specific **sites** for restriction **endonuclease I** -**SceI** (a Saccharomyces cerevisiae **intron**-encoded rare-cutter **endonuclease** recognizing 18-bp sequence) and cDNA encoding **I-SceI**. The **I-SceI** **site** is recognized and cleaved in vivo to relase the repair matrix and induce homologous recombination. The method has applications in treating or prophylaxis of a genetic disease in an individual in need.

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| PI WO 2000046385 | A1 | 20000810 | WO 2000-US2949 | 20000203 |
| W: AU, CA, JP, US | | | | |
| RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE | | | | |
| EP 1151124 | A1 | 20011107 | EP 2000-908491 | 20000203 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI | | | | |
| JP 2002535994 | T2 | 20021029 | JP 2000-597444 | 20000203 |
| US 2002110898 | A1 | 20020815 | US 2001-922495 | 20010803 |

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     FILE 'MEDLINE, AGRICOLA, CANCERLIT, SCISEARCH, CAPLUS, MEDICONF' ENTERED
     AT 18:14:02 ON 05 FEB 2003
L1         2908 S I-SCE? OR I-CSM? OR I-PAN? OR I-CEUI OR I-PPO? OR I-CRE? OR I
L2          829 S L1 AND SITE
L3          647 S L2 AND ENDO?
L4          287 S L3 AND INTRON
L5          125 DUP REM L4 (162 DUPLICATES REMOVED)
L6          125 FOCUS L5 1-
L7           29 S L5 AND CHROMOSOME
L8           29 SORT L7 PY
L9           12 S L8 AND (MAMMAL? OR ANIMAL)
L10         387 S L1 AND CHROMOSOME
L11         111 S L10 AND (ANIMAL OR MAMMAL?)
L12          55 DUP REM L11 (56 DUPLICATES REMOVED)
L13          55 FOCUS L12 1-
L14        2451 S I-CSM? OR I-PAN? OR I-CEU? OR I-PPO? OR I-CRE? OR I-TEV?
L15         209 S L14 AND CHROMOSOME
L16          11 S L15 AND (MAMMAL? OR ANIMAL?)
L17           9 DUP REM L16 (2 DUPLICATES REMOVED)
L18           9 SORT L17 PY
L19         168 S L14 AND RECOMB?
L20         100 DUP REM L19 (68 DUPLICATES REMOVED)
L21         100 FOCUS L20 1-
L22        2930 S L1 OR L14
L23        1761 S L22 AND PY<=1995
L24         238 S L23 AND (ANIMAL OR MAMMALIAN OR EUKARY?)
L25         196 DUP REM L24 (42 DUPLICATES REMOVED)
L26         196 FOCUS L25 1-
L27         512 S L22 AND (ANIMAL OR MAMMALIAN OR EUKARY?)
L28         287 S L27 AND (SITE OR RECOMB? OR GENO?)
L29         157 DUP REM L28 (130 DUPLICATES REMOVED)
L30         157 FOCUS L29 1-
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(FILE 'HOME' ENTERED AT 18:13:53 ON 05 FEB 2003)

FILE 'MEDLINE, AGRICOLA, CANCERLIT, SCISEARCH, CAPLUS, MEDICONF' ENTERED AT 18:14:02 ON 05 FEB 2003

L1 2908 S I-SCE? OR I-CSM? OR I-PAN? OR I-CEUI OR I-PPO? OR I-CRE? OR I
L2 829 S L1 AND SITE
L3 647 S L2 AND ENDO?
L4 287 S L3 AND INTRON
L5 125 DUP REM L4 (162 DUPLICATES REMOVED)
L6 125 FOCUS L5 1-
L7 29 S L5 AND CHROMOSOME
L8 29 SORT L7 PY
L9 12 S L8 AND (MAMMAL? OR ANIMAL)
L10 387 S L1 AND CHROMOSOME
L11 111 S L10 AND (ANIMAL OR MAMMAL?)
L12 55 DUP REM L11 (56 DUPLICATES REMOVED)
L13 55 FOCUS L12 1-

=> d an ti so au ab pi l13 1-9

L13 ANSWER 1 OF 55 CAPLUS COPYRIGHT 2003 ACS
AN 2002:403935 CAPLUS
DN 136:396983
TI Nucleotide sequence encoding yeast restriction endonuclease **I-SceI** and uses in genetic mapping and site-directed gene recombination
SO U.S., 84 pp., Cont.-in-part of U.S. 5,792,632.
CODEN: USXXAM
IN Dujon, Bernard; Choulika, Andre; Perrin, Arnaud; Nicolas, Jean-Francois
AB The present invention relates to an isolated yeast DNA encoding the restriction endonuclease **I-SceI**, and use of **I-SceI** for mapping eukaryotic genomes and for in vivo site directed genetic recombination. Specifically, the invention relates to a vector comprising a plasmid, bacteriophage, or cosmid vector contg. the DNA sequence of the enzyme **I-SceI**. The invention also relates to E. coli, eukaryotic cells transformed with a vector of the invention, transgenic **animal** with the DNA sequence encoding **I-SceI**. The invention relates to a transgenic organism in which at least one restriction site for the enzyme **I-SceI** has been inserted in a **chromosome** of the organism. The invention further relates to methods for gene mapping in yeast **chromosome**, yeast artificial **chromosome**, and cosmids, and site-directed insertion of genes.

| | PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|---|
| PI | US 6395959 | B1 | 20020528 | US 1996-643732 | 19960506 |
| | US 5474896 | A | 19951212 | US 1992-971160 | 19921105 |
| | US 5792632 | A | 19980811 | US 1994-336241 | 19941107 |

L13 ANSWER 2 OF 55 CAPLUS COPYRIGHT 2003 ACS
AN 1998:545391 CAPLUS
DN 129:172448
TI Cloning and expression of gene for restriction endonuclease **I-SceI** of Saccharomyces cerevisiae and use of **I-SceI**
SO U.S., 79 pp., Cont.-in-part of U. S. 5,474,896.
CODEN: USXXAM
IN Dujon, Bernard; Choulika, Andre; Perrin, Arnaud; Nicolas, Jean-francois
AB A mitochondrial gene encoding restriction endonuclease **I-SceI** of Saccharomyces cerevisiae and a synthetic universal code encoding **I-SceI** for the expression in Escherichia coli and yeast are provided. Applications of **I-SceI** for genetically mapping yeast **chromosomes** by the nested chromosomal fragmentation strategy, inducing double stranded DNA break, and in vivo site-directed insertion of genes and homologous recombination in eukaryotes are also described. It may also be used for prepg. transgenic **animal** models of human diseases and genetic disorders.

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|

L26 ANSWER 6 OF 196 CAPLUS COPYRIGHT 2003 ACS
AN 1995:346854 CAPLUS
DN 122:98806
TI Transformation vectors that direct the integration of transforming DNA into the ribosomal DNA of a **eukaryotic** host
SO PCT Int. Appl., 35 pp.
CODEN: PIXXD2
IN Jacobs, Eric
AB Transposition cassettes that preferably integrate into the ribosomal DNA of a **eukaryotic** host and based on a **eukaryotic** transposable element are described for use in gene therapy. The vectors carrying these cassettes also carry all the functions necessary for integration. The construction of a cassette for integration of transforming DNA into the human 28 S rRNA gene using the mobile intron 3 of the Carolina strain of Physarum polycephalum is demonstrated. This cassette was then introduced into an adenovirus that also carried an expression cassette for the P. polycephalum mobility endonuclease **I-Ppo-I**. A neomycin resistance marker was also included in the constructs.

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE | |
|---|---|---|---|---|---|
| PI WO 9424300 | A1 | 19941027 | WO 1994-FR419 | 19940414 | <-- |
| W: AU, CA, JP, US | | | | | |
| RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE | | | | | |
| FR 2703996 | A1 | 19941021 | FR 1993-4530 | 19930416 | <-- |
| FR 2703996 | B1 | 19950721 | | | |
| CA 2160697 | AA | 19941027 | CA 1994-2160697 | 19940414 | <-- |
| AU 9465719 | A1 | 19941108 | AU 1994-65719 | 19940414 | <-- |
| AU 686156 | B2 | 19980205 | | | |
| EP 694072 | A1 | 19960131 | EP 1994-913647 | 19940414 | |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE | | | | | |
| JP 08508878 | T2 | 19960924 | JP 1994-522836 | 19940414 | |
| US 6346414 | B1 | 20020212 | US 1995-532657 | 19951016 | |

30 ANSWER 6 OF 157 CAPLUS COPYRIGHT 2003 ACS
AN 1994:70296 CAPLUS
DN 120:70296
TI Retroviral gene transfer vectors containing single cleavage **sites** for mapping **mammalian genomes**
SO Methods in Molecular Genetics (1993), 2(Gene and Chromosome Analysis, Pt. B), 67-77
CODEN: MEMGE6; ISSN: 1067-2389
AU Kurdi-Haidar, Buran; Friedmann, Theodore
AB A review with 18 refs. Topics include: prepn. of chromosomal DNA in agarose microbeads, single-**site** Achilles heel cleavage of **mammalian** DNA, endonuclease **I-SceI** single-**site** cleavage.

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|---|---|---|---|---|---|
| PI | US 5792632 | A | 19980811 | US 1994-336241 | 19941107 |
| | US 5474896 | A | 19951212 | US 1992-971160 | 19921105 |
| | US 5866361 | A | 19990202 | US 1995-465273 | 19950605 |
| | CA 2203569 | AA | 19960517 | CA 1995-2203569 | 19951106 |
| | WO 9614408 | A2 | 19960517 | WO 1995-EP4351 | 19951106 |
| | WO 9614408 | A3 | 19960829 | | |

W: CA, JP
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

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|---|---|---|---|---|
| EP 791058 | A1 | 19970827 | EP 1995-938418 | 19951106 |

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE

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|---|---|---|---|---|
| JP 10508478 | T2 | 19980825 | JP 1995-515058 | 19951106 |
| US 6395959 | B1 | 20020528 | US 1996-643732 | 19960506 |
| US 5948678 | A | 19990907 | US 1998-119024 | 19980720 |

L13 ANSWER 3 OF 55 CAPLUS COPYRIGHT 2003 ACS
AN 1996:428575 CAPLUS
DN 125:107019
TI Nucleotide sequence encoding yeast enzyme **I-SceI** and its use in inducing homologous recombination in eukaryotic cells and protein production in transgenic **animals**
SO PCT Int. Appl., 122 pp.
CODEN: PIXXD2
IN Choulika, Andre; Perrin, Arnaud; Dujon, Bernard; Nicolas, Jean-Francois
AB Synthetic DNA encoding the enzyme **I-SceI** is provided. The DNA sequence can be incorporated in cloning and expression vectors, transformed cell lines and transgenic **animals**. The vectors are useful in gene mapping and site-directed insertion of genes. A synthetic gene encoding Saccharomyces cerevisiae **I-SceI** restriction endonuclease was expressed in Escherichia coli and yeast. The enzyme was used in genetic mapping of a yeast **chromosome**, of YAC's, and of cosmids. **I-SceI** efficiently induced double-stranded breaks in a chromosomal target in **mammalian** cells and the breaks were repaired using a donor mol. that shares homol. with the regions flanking the break.

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| PI WO 9614408 | A2 | 19960517 | WO 1995-EP4351 | 19951106 |
| WO 9614408 | A3 | 19960829 | | |

W: CA, JP
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

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|---|---|---|---|---|
| US 5792632 | A | 19980811 | US 1994-336241 | 19941107 |
| EP 791058 | A1 | 19970827 | EP 1995-938418 | 19951106 |

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE

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|---|---|---|---|---|
| JP 10508478 | T2 | 19980825 | JP 1995-515058 | 19951106 |

L13 ANSWER 4 OF 55 CANCERLIT
AN 96605697 CANCERLIT
TI Repair of DNA double strand breaks in **mammalian** cells by homologous recombination and end-joining mechanisms (Meeting abstract).
SO J Cell Biochem, (1995) Suppl 21A 328.
ISSN: 0730-2312.
AU Jasin M; Rouet P; Smih F
AB To study the repair of DSBs introduced into **mammalian** chromosomal DNA, we have developed expression vectors for rare-cutting, site-specific endonucleases from S cerevisiae. We used the universal code equivalent of the mitochondrial intron-encoded endonuclease **I-Sce** I to build the **mammalian** expression vector, pCMV-**I-Sce** I. The **I-Sce** I sequence was provided by B Dujon, Pasteur Institute. In addition to providing a consensus Kozak sequence for efficient translation, the **I-Sce** I ORF was modified by fusing sequences encoding a nuclear localization signal and a hemagglutinin epitope tag. Our initial assay for in vivo cutting and enhanced recombination measures extrachromosomal recombination, since this form of recombination is very efficient in **mammalian** cells and sensitive to DSBs. The assay utilizes RSVCAT plasmid substrates consisting of overlapping chloramphenicol acetyltransferase (CAT) gene fragments transiently transfected into cells. The RSVCAT plasmids were modified by the insertion of a synthetic **I-Sce** I site at the end of the homology repeats and cotransfections were carried out in COS 1 cells. We observed a substantial

increase of CAT activity in cotransfections of pCMV-**I-Sce** I with CAT substrates containing the **I-Sce** I site but not with plasmids lacking the site. Southern analysis verified in vivo cleavage, as well as recombination. Constitutive expression of the endonuclease is not toxic to mouse 3T3 cells. These results have been recently published (Rouet P et al, Proc Natl Acad Sci; 91:6064 1994). We have recently observed efficient cutting of introduced chromosomal **I-Sce** I sites in 3T3 cells and have found that they are recombinogenic, stimulating gene targeting two to three orders of magnitude. However, they are also repaired efficiently by end-joining mechanisms (Rouet P et al, Mol Cell Biol, in press). Results of these studies were presented. It is expected that expression of rare-cutting endonucleases in **mammalian** cells will provide a powerful tool for the analysis of the repair of chromosomal DSBs, as well as for molecular genetic manipulations, such as **chromosome** fragmentation and, potentially, gene-targeting.

L13 ANSWER 5 OF 55 CAPLUS COPYRIGHT 2003 ACS
AN 2001:527625 CAPLUS
DN 136:227704
TI Recombination between two **chromosomes**: Implications for genomic integrity in **mammalian** cells
SO Cold Spring Harbor Symposia on Quantitative Biology (2000), 65, 553-560
CODEN: CSHSAZ; ISSN: 0091-7451
AU Richardson, C.; Jasin, M.
AB A mouse embryonic stem (ES) cell system was used to evaluate the potential of two double-strand breaks (DSBs) to result in genome rearrangements when the homologous sequences are in opposite orientation relative to the centromere. The defective neomycin phosphotransferase (neo) gene substrates were inserted into loci on two heterologous **chromosomes** in mouse ES cells. Each neo gene is defective because the 18-bp **I-SceI** site was inserted within the neo-coding region. The reverse/N2 cell line had the neo sequences in opposite orientation, such that the S2neo gene is transcribed away from the centromere and the N2neo gene is transcribed toward the centromere. The results indicated that the **mammalian** cell is capable of searching the genome and finding homologous sequences suitable for DSB repair, even when these sequences reside on heterologous **chromosomes**. Homologous recombination was a major pathway for the repair of DSBs. Template choice for homologous repair, the no. of DSBs within the cell, and the relative orientation of interacting **chromosomes** appeared to affect the repair mechanisms used during recombinatorial repair and ultimately play a part in maintaining genome stability.

L13 ANSWER 6 OF 55 MEDLINE
AN 95140628 MEDLINE
TI Repair of a specific double-strand break generated within a **mammalian chromosome** by yeast endonuclease **I-SceI**.
SO NUCLEIC ACIDS RESEARCH, (1994 Dec 25) 22 (25) 5649-57.
Journal code: 0411011. ISSN: 0305-1048.
AU Lukacsovich T; Yang D; Waldman A S
AB We established a mouse Ltk- cell line that contains within its genome a herpes simplex virus thymidine kinase gene (tk) that had been disrupted by the insertion of the recognition sequence for yeast endonuclease **I-SceI**. The artificially introduced 18 bp **I-SceI** recognition sequence was likely a unique sequence in the genome of the mouse cell line. To assess whether an induced double-strand break (DSB) in the genomic tk gene would be repaired preferentially by gene targeting or non-homologous recombination, we electroporated the mouse cell line with endonuclease **I-SceI** alone, one of two different gene targeting constructs alone, or with **I-SceI** in conjunction with each of the two targeting constructs. Each targeting construct was, in principle, capable of correcting the defective genomic tk sequence via homologous recombination. tk+ colonies were recovered following electroporation of cells with **I-SceI** in the presence or absence of a targeting construct. Through the detection of small deletions at the **I-SceI** recognition sequence in the mouse genome, we present evidence that a specific DSB can be introduced into the genome of a living

**mammalian** cell by yeast endonuclease **I-SceI**.
We further report that a DSB in the genome of a mouse Ltk- cell is repaired preferentially by non-homologous end-joining rather than by targeted homologous recombination with an exogenous donor sequence. The potential utility of this system is discussed.

L13 ANSWER 7 OF 55 MEDLINE
AN 95198715 MEDLINE
TI Induction of homologous recombination in **mammalian chromosomes** by using the **I-SceI** system of Saccharomyces cerevisiae.
SO MOLECULAR AND CELLULAR BIOLOGY, (1995 Apr) 15 (4) 1968-73.
Journal code: 8109087. ISSN: 0270-7306.
AU Choulika A; Perrin A; Dujon B; Nicolas J F
AB The mitochondrial intron-encoded endonuclease **I-SceI** of Saccharomyces cerevisiae has an 18-bp recognition sequence and, therefore, has a very low probability of cutting DNA, even within large genomes. We demonstrate that double-strand breaks can be initiated by the **I-SceI** endonuclease at a predetermined location in the mouse genome and that the breaks can be repaired with a donor molecule homologous regions flanking the breaks. This induced homologous recombination is approximately 2 orders of magnitude more frequent than spontaneous homologous recombination and at least 10 times more frequent than random integration near an active promoter. As a consequence of induced homologous recombination, a heterologous novel sequence can be inserted at the site of the break. This recombination can occur at a variety of chromosomal targets in differentiated and multipotential cells. These results demonstrate homologous recombination involving chromosomal DNA by the double-strand break repair mechanism in **mammals** and show the usefulness of very rare cutter endonucleases, such as **I-SceI**, for designing genome rearrangements.

L13 ANSWER 8 OF 55 CAPLUS COPYRIGHT 2003 ACS
AN 2002:575221 CAPLUS
DN 137:136055
TI Combinatorial expression libraries with individual members of the library containing concatemers of expression cassettes
SO PCT Int. Appl., 124 pp.
CODEN: PIXXD2
IN Goldsmith, Neil; Sorensen, Alexandra M. P. Santana; Nielsen, Soren V. S.; Naesby, Michael
AB Combinatorial gene expression libraries in which individual clones contain large nos. of expression cassettes and methods of constructing such libraries are described. Each member of the library contains a large no. of expression cassettes that are randomly selected from a pool of cassettes during the construction of the library. Individual expression cassettes are flanked by a common pair of restriction sites and have the same promoter and terminator for uniform regulation of expression of the cloned inserts. The library of concatemers is created from a library of individual clones. This primary library, typically a cDNA library, has the individual cassette and its flanking restriction sites flanked by a second pair of restriction sites. The cassettes are released from the library and ligated into concatemers that are then cloned into a vector capable of stabilizing large inserts, esp. artificial **chromosomes**. The variability within the combinatorial library can be increased by using cDNA libraries from multiple sources. Such libraries are useful in discovery of novel or modified metabolic pathways leading to the prodn. of novel compds. for e.g. drug discovery and to the prodn. of known compds. in novel quantities or in novel compartments of the cells. The expression libraries in particular are composed of host cells capable of coordinated and controllable expression of large nos. of heterologous genes in the host cells.

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| PI WO 2002059296 | A2 | 20020801 | WO 2002-DK55 | 20020125 |

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ,

UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

L13 ANSWER 9 OF 55 CAPLUS COPYRIGHT 2003 ACS
AN 1997:595160 CAPLUS
DN 127:289030
TI Creation and removal of embedded ribonucleotides in chromosomal DNA during **mammalian** Okazaki fragment processing
SO Journal of Biological Chemistry (1997), 272(36), 22591-22599
CODEN: JBCHA3; ISSN: 0021-9258
AU Rumbaugh, Jeffrey A.; Murante, Richard S.; Shi, Shuying; Bambara, Robert A.
AB **Mammalian** RNase HI has been shown to specifically cleave the initiator RNA of Okazaki fragments at the RNA-DNA junction, leaving a single ribonucleotide attached to the 5'-end of the downstream DNA segment. This monoribonucleotide can then be removed by the **mammalian** 5'- to 3'-exo-/endonuclease, a RAD2 homolog-1 (RTH-1) class nuclease, also known as flap endonuclease-1 (FEN-1). Although FEN-1/RTH-1 nuclease often requires an upstream primer for efficient activity, the presence of an upstream primer is usually inhibitory or neutral for removal of this 5'-monoribonucleotide. Using model Okazaki fragment substrates, we found that DNA ligase I can seal a 5'-monoribonucleotide into DNA. When both ligase and FEN-1/RTH-1 were present simultaneously, some of the 5'-monoribonucleotides were ligated into DNA, while others were released. Thus, a 5'-monoribonucleotide, particularly one that is made resistant to FEN-1/RTH-1-directed cleavage by extension of an inhibitory upstream primer, can be ligated into the **chromosome**, despite the presence of FEN-1/RTH-1 nuclease. DNA ligase I was able to seal different monoribonucleotides into the DNA for all substrates tested, with an efficiency of 1-13% that of ligating DNA. These embedded monoribonucleotides can be removed by the combined action of RNase HI, cutting on the 5'-side, and FEN-1/RTH-1 nuclease, cleaving on the 3'-side. After FEN-1/RTH-1 action and extension by polymn., DNA ligase I can join the entirely DNA strands to complete repair.

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L30 ANSWER 5 OF 157 CAPLUS COPYRIGHT 2003 ACS
AN 1998:545391 CAPLUS
DN 129:172448
TI Cloning and expression of gene for restriction endonuclease **I-SceI** of Saccharomyces cerevisiae and use of **I-SceI**
SO U.S., 79 pp., Cont.-in-part of U. S. 5,474,896.
CODEN: USXXAM
IN Dujon, Bernard; Choulika, Andre; Perrin, Arnaud; Nicolas, Jean-francois
AB A mitochondrial gene encoding restriction endonuclease **I-SceI** of Saccharomyces cerevisiae and a synthetic universal code encoding **I-SceI** for the expression in Escherichia coli and yeast are provided. Applications of **I-SceI** for genetically mapping yeast chromosomes by the nested chromosomal fragmentation strategy, inducing double stranded DNA break, and in vivo **site**-directed insertion of genes and homologous **recombination** in **eukaryotes** are also described. It may also be used for prepg. transgenic **animal** models of human diseases and genetic disorders.

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| PI US 5792632 | A | 19980811 | US 1994-336241 | 19941107 |
| US 5474896 | A | 19951212 | US 1992-971160 | 19921105 |
| US 5866361 | A | 19990202 | US 1995-465273 | 19950605 |
| CA 2203569 | AA | 19960517 | CA 1995-2203569 | 19951106 |
| WO 9614408 | A2 | 19960517 | WO 1995-EP4351 | 19951106 |
| WO 9614408 | A3 | 19960829 | | |
| W: CA, JP | | | | |
| RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE | | | | |
| EP 791058 | A1 | 19970827 | EP 1995-938418 | 19951106 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE | | | | |
| JP 10508478 | T2 | 19980825 | JP 1995-515058 | 19951106 |
| US 6395959 | B1 | 20020528 | US 1996-643732 | 19960506 |
| US 5948678 | A | 19990907 | US 1998-119024 | 19980720 |

30 ANSWER 7 OF 157 CAPLUS COPYRIGHT 2003 ACS
AN 1996:428575 CAPLUS
DN 125:107019
TI Nucleotide sequence encoding yeast enzyme **I-SceI** and its use in inducing homologous **recombination** in **eukaryotic** cells and protein production in transgenic **animals**
SO PCT Int. Appl., 122 pp.
CODEN: PIXXD2
IN Choulika, Andre; Perrin, Arnaud; Dujon, Bernard; Nicolas, Jean-Francois
AB Synthetic DNA encoding the enzyme **I-SceI** is provided. The DNA sequence can be incorporated in cloning and expression vectors, transformed cell lines and transgenic **animals**. The vectors are useful in gene mapping and **site**-directed insertion of genes. A synthetic gene encoding Saccharomyces cerevisiae **I-SceI** restriction endonuclease was expressed in Escherichia coli and yeast. The enzyme was used in genetic mapping of a yeast chromosome, of YAC's, and of cosmids. **I-SceI** efficiently induced double-stranded breaks in a chromosomal target in **mammalian** cells and the breaks were repaired using a donor mol. that shares homol. with the regions flanking the break.

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| PI WO 9614408 | A2 | 19960517 | WO 1995-EP4351 | 19951106 |
| WO 9614408 | A3 | 19960829 | | |
| W: CA, JP | | | | |
| RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE | | | | |
| US 5792632 | A | 19980811 | US 1994-336241 | 19941107 |
| EP 791058 | A1 | 19970827 | EP 1995-938418 | 19951106 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE | | | | |
| JP 10508478 | T2 | 19980825 | JP 1995-515058 | 19951106 |

L32 ANSWER 1 OF 1 CANCERLIT
AN 96605697 CANCERLIT
DN 96605697
TI Repair of DNA double strand breaks in **mammalian** cells by homologous **recombination** and end-joining mechanisms (Meeting abstract).
AU Jasin M; Rouet P; Smih F
CS Cell Biology and Genetics Program, Memorial Sloan-Kettering Inst., 1275 York Ave., New York, NY 10021.
SO J Cell Biochem, (1995) Suppl 21A 328.
ISSN: 0730-2312.
DT (MEETING ABSTRACTS)
LA English
FS Institute for Cell and Developmental Biology
EM 199605
ED Entered STN: 19970509
Last Updated on STN: 19970509
AB To study the repair of DSBs introduced into **mammalian** chromosomal DNA, we have developed expression vectors for rare-cutting, **site**-specific endonucleases from S cerevisiae. We used the universal code equivalent of the mitochondrial intron-encoded endonuclease **I-Sce** I to build the **mammalian** expression vector, pCMV-**I-Sce** I. The **I-Sce** I sequence was provided by B **Dujon**, Pasteur Institute. In addition to providing a consensus Kozak sequence for efficient translation, the **I-Sce** I ORF was modified by fusing sequences encoding a nuclear localization signal and a hemagglutinin epitope tag. Our initial assay for in vivo cutting and enhanced **recombination** measures extrachromosomal **recombination**, since this form of **recombination** is very efficient in **mammalian** cells and sensitive to DSBs. The assay utilizes RSVCAT plasmid substrates consisting of overlapping chloramphenicol acetyltransferase (CAT) gene fragments transiently transfected into cells. The RSVCAT plasmids were modified by the insertion of a synthetic **I-Sce** I **site** at the end of the homology repeats and cotransfections were carried out in COS 1 cells. We observed a substantial increase of CAT activity in cotransfections of pCMV-**I-Sce** I with CAT substrates containing the **I-Sce** I **site** but not with plasmids lacking the **site**. Southern analysis verified in vivo cleavage, as well as **recombination**. Constitutive expression of the endonuclease is not toxic to mouse 3T3 cells. These results have been recently published (Rouet P et al, Proc Natl Acad Sci; 91:6064 1994). We have recently observed efficient cutting of introduced chromosomal **I-Sce** I **sites** in 3T3 cells and have found that they are **recombinogenic**, stimulating gene targeting two to three orders of magnitude. However, they are also repaired efficiently by end-joining mechanisms (Rouet P et al, Mol Cell Biol, in press). Results of these studies were presented. It is expected that expression of rare-cutting endonucleases in **mammalian** cells will provide a powerful tool for the analysis of the repair of chromosomal DSBs, as well as for molecular genetic manipulations, such as chromosome fragmentation and, potentially, gene-targeting.
RN 9007-49-2 (DNA)
CN EC 2.3.1.28 (Chloramphenicol O-Acetyltransferase); EC 3.1.- (Endonucleases); 0 (Plasmids)